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Allele-selective SNP editing utilizing AAV5-delivered Life Edit® nuclease and guide RNA resulting in meaningful reduction of mutant HTT protein

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18TH ANNUAL HUNTINGTON'S DISEASE THERAPEUTICS CONFERENCE
DUBROVNIK, CROATIA | 25TH APRIL 2023

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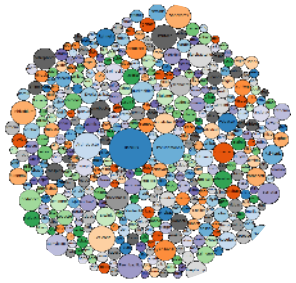
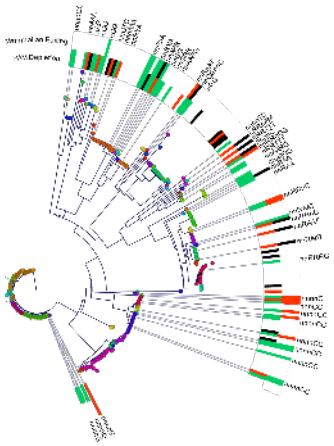
Evolutionarily Distant Nucleases With a Diversity of PAM Sequences

90,000+



Large sequenced proprietary microbe collection

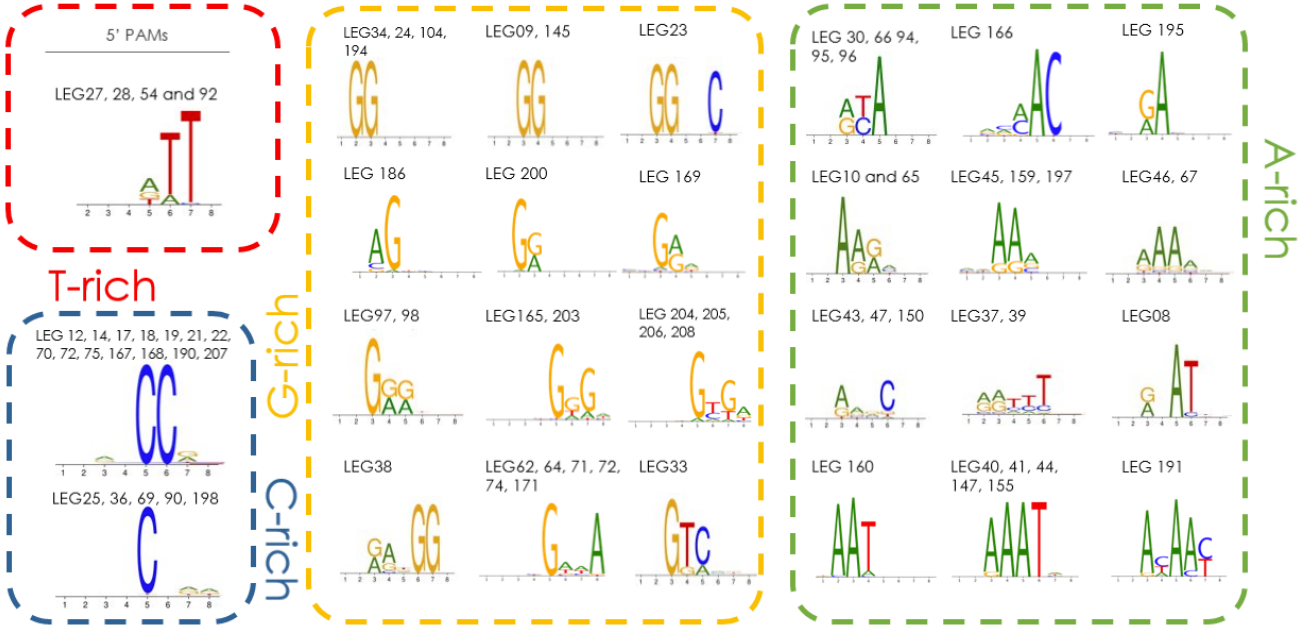


Bioinformatic Identification of 1000's of unique CRISPR-Cas systems

Focus:

Type II
Type V

Single protein effectors targeting DNA



Collection of RGNs with diverse PAM sequences

Exclusive access to the genome editing systems identified in the continuously expanding AgBiome proprietary collection for use in human therapeutics

Collection enables the ability to **find additional enzymatic activities** to build future editing systems (e.g., proprietary base editors, transposases, others)

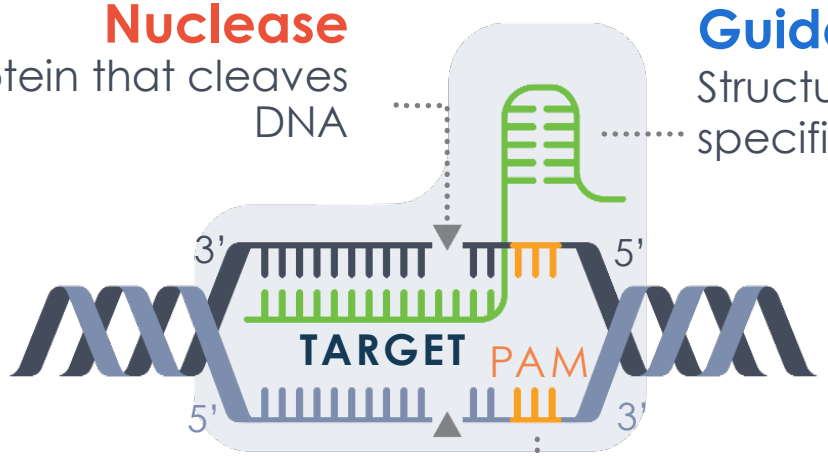
Life Edit Gene Editing Technology & Allele-Specific Editing in HD

Life Edit's Collection of Proprietary RNA guided Nucleases Enable Targeting of Disease-Linked Genes

CRISPR System Components

Nuclease
Protein that cleaves DNA

Guide RNA
Structure determines targeting specificity of the nuclease



PAM
Recognition sequence required for cutting

Three components of a CRISPR system:

- Nuclease
- Guide RNA
- Protospacer Adjacent Motif (PAM)

Life Edit has a library of nucleases (Life Edit Genes or LEGs) with unique PAM recognition sequences, enabling us to target diverse genomic sequences

Life Edit Lead Nucleases

Nuclease	Base Pairs	Amino Acids	MW kDa	PAM
LEG-A	3213	1071	126	NNNNCC
LEG-B	3450	1150	133	NNRYA
LEG-C	3156	1052	124	NNGRR
LEG-D	3390	1130	130	NNGG
SpCas9	4104	1368	158	NGG

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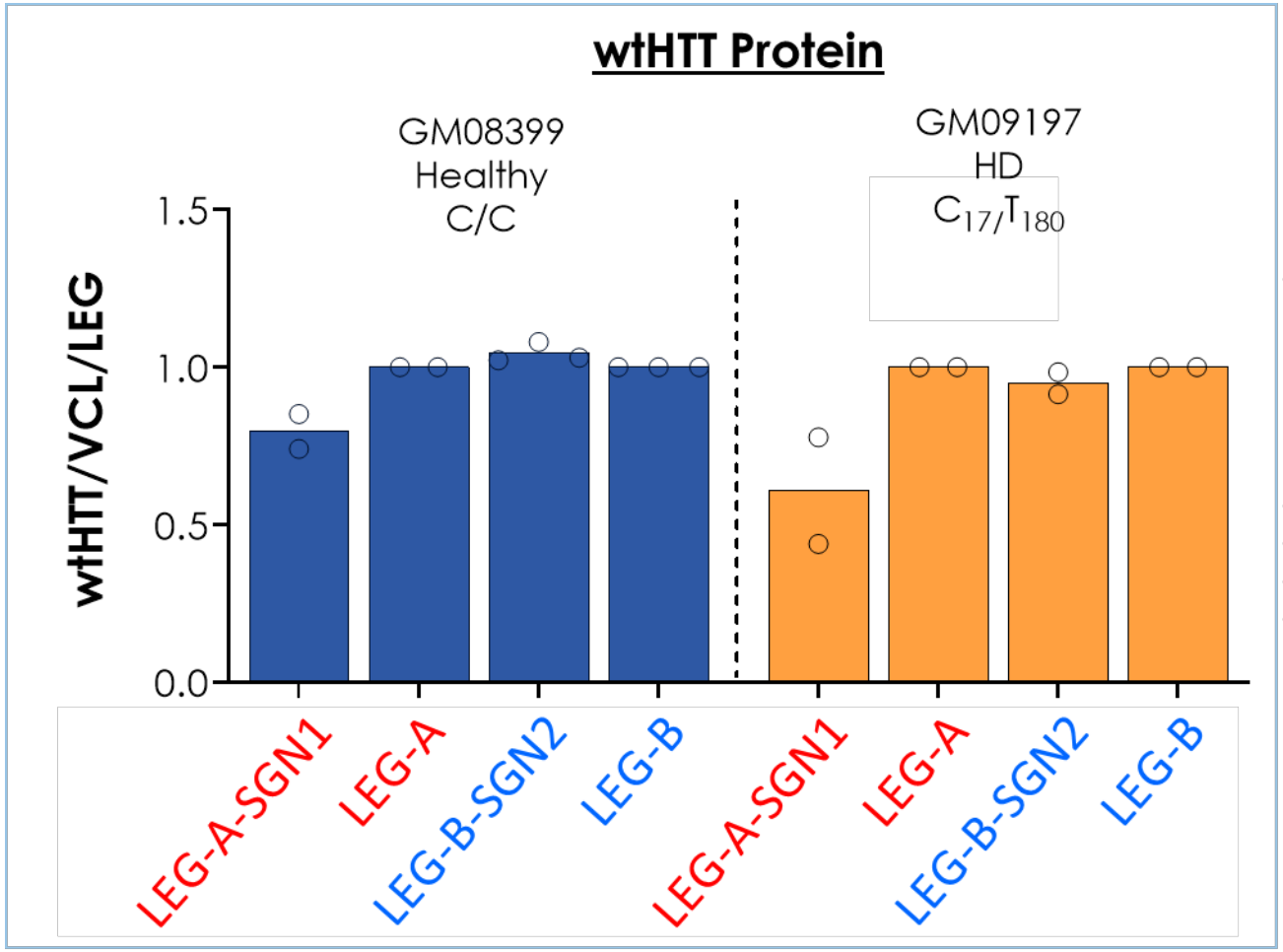
Allele Selective Editing of Exon50 rs362331 in Patient Fibroblasts



- Cell Lines
  - Patient fibroblast line(GM09197) is heterozygous (C/T) for Exon50 rs362331
    - T-allele expresses mutHTT protein
    - C-allele expresses wtHTT protein
  - Healthy patient fibroblast line GM08399 is homozygous for Exon50 rs362331
    - Both alleles carry 'C' & express wtHTT protein
- RNA nucleofection
- 96hrs timepoint
- Jess capillary electrophoresis protein quantification system
- EPR5526 antibody (recognizes mutHTT and wtHTT protein)

- **LEG-B-SGN2** targeting 'T' SNP does not affect wtHTT protein in fibroblast line derived from healthy donor that is homozygous (C/C) for rs362331
- **LEG-B-SGN2 targeting 'T' SNP does not affect wtHTT protein in patient-derived fibroblast line that is heterozygous (C/T) for rs362331 with CAG repeat expansion in-phase with the 'T' allele**

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Therapeutic Strategy for Huntington's Disease

Target Tissue	Striatum Medium Spiny Neurons
Delivery Vector	AAV5
Route of Admin.	Intrastriatal
Genetic Target	HTT Exon50 SNP 362331
Editing Modality	DSB → NHEJ
Nuclease	LEG-A ('C') Secondary target LEG-B ('T') Lead target

Huntingtin Gene

SNP rs362331 'C' or 'T'

SNP Allele-selective Editing	The PAM site generated by HTT Exon50 rs362331 SNP allows selective targeting of HTT alleles with Life Edit nucleases based on the presence of 'C' (LEG-A) or 'T' (LEG-B) nucleotide
Therapeutic Goal	AAV5-delivered Life Edit nuclease (LEG-A or LEG-B) and guide RNA (*SGN1* or SGN2) targeting Huntingtin's Exon50 rs362331 *'C'* or 'T' SNP, to be **expressed in at least 50% of striatal neurons**, resulting in **>40% knockdown of mutant HTT protein**
Mode of Action	Allele-specific DSBs activate NHEJ repair pathway, form INDELs that cause frameshift leading to mRNA containing premature-stop codons which are degraded by non-sense mediated decay

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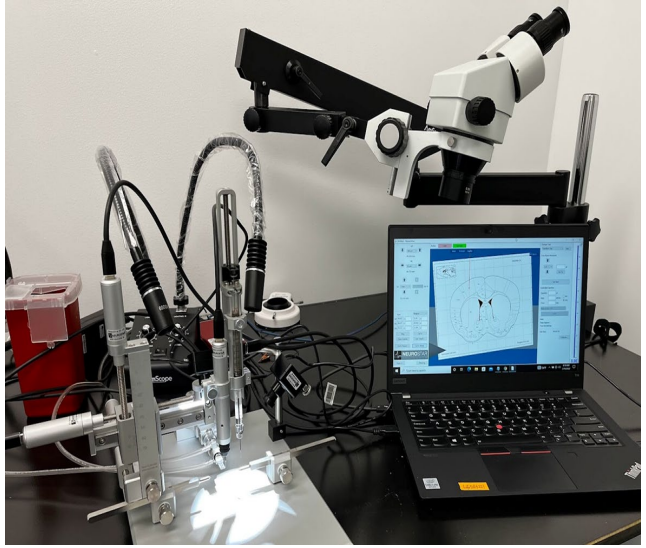
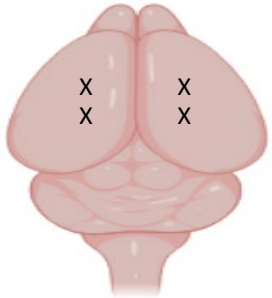
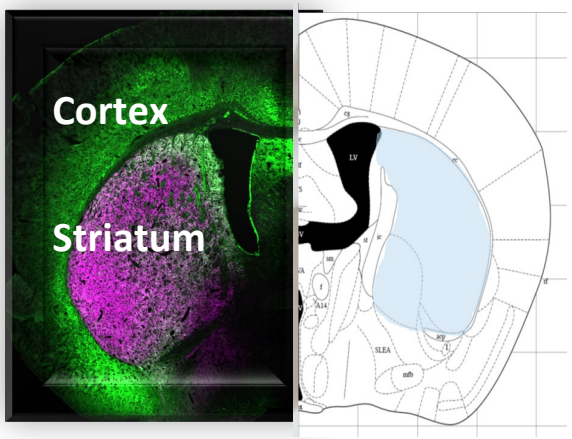
Intrastriatal Administration Qualification





Striatal Injection Sites

2µl/site



AAV5-eGFP
DARPP32 (MSNs)

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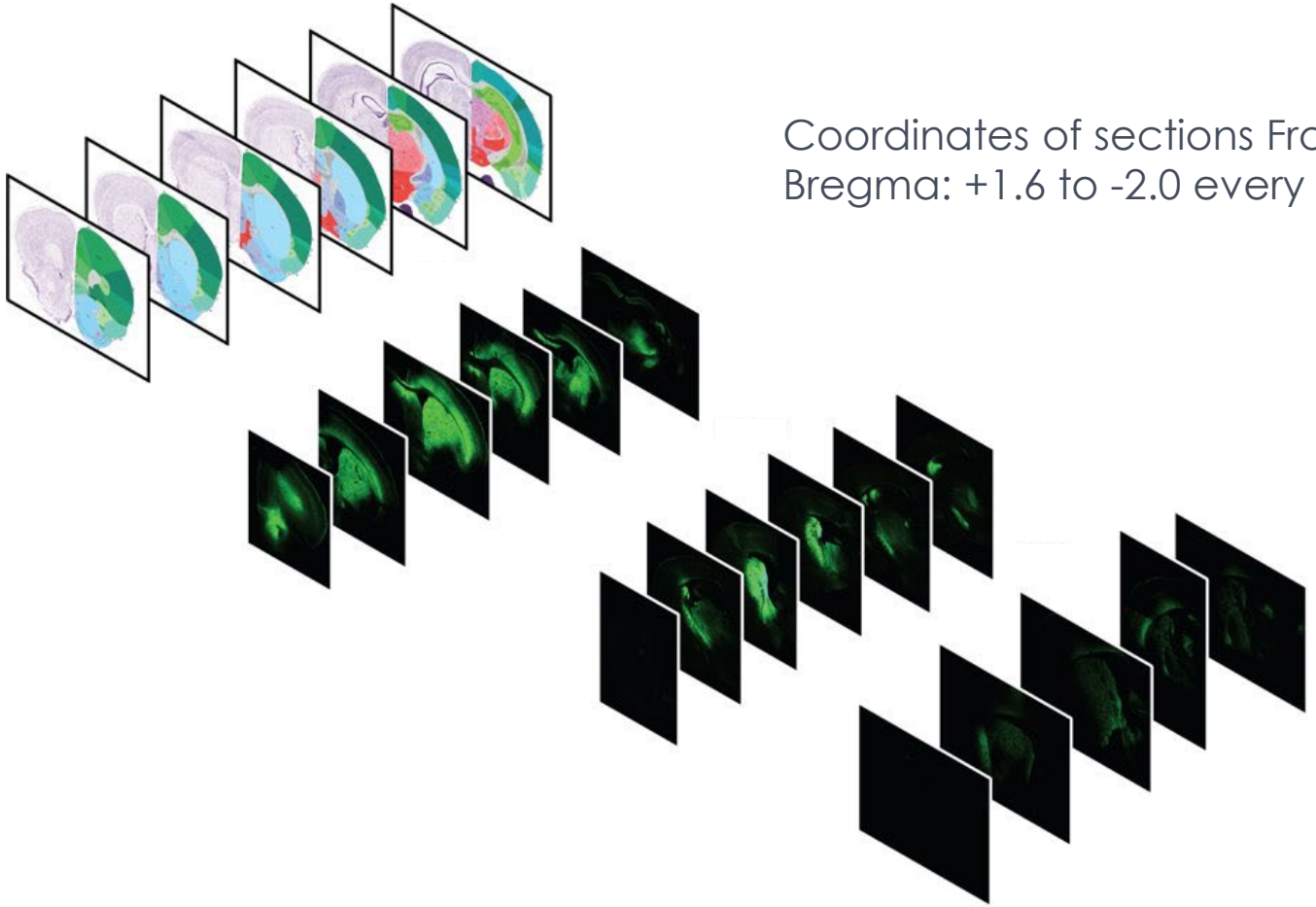
Transgene Promoter Expression and Biodistribution; Transgene Expression Optimization

Identification of promoters with expressed in at least 50% of striatal neurons



Coordinates of sections From Bregma: +1.6 to -2.0 every 400 µm

All images were minimally enhanced (Exposure +3; Brightness +100) on Ai for visualization purposes

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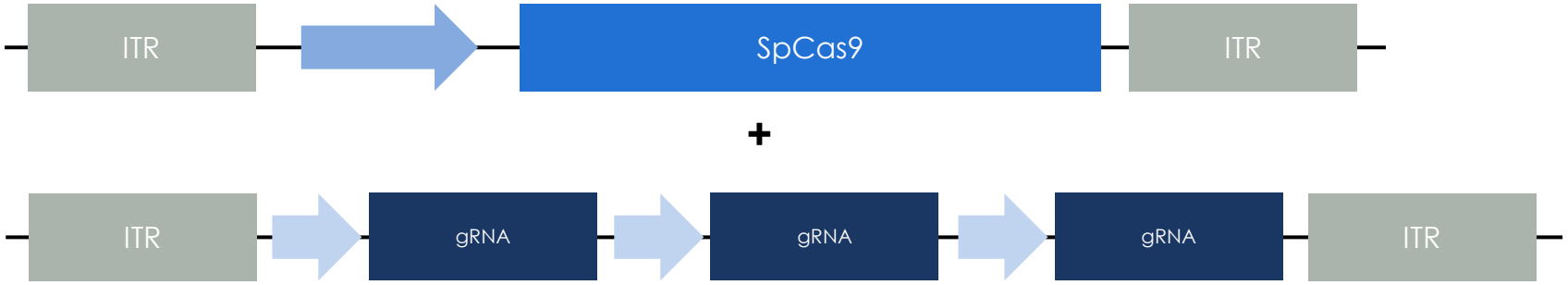
Small Nucleases Enable Single AAV Delivery

SpCas9 vector + sgRNA vector



- Multiple sgRNAs can be used
- Must purify two AAVs for co-infection

LEG-A or LEG-B/sgRNA

- Use of LEG-A or LEG-B adds >1kb of space for other elements

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Allele Selective Editing of HTT SNP rs362331

AAV5-LEG-A Targeting 'C' allele

Target

AAV5-delivered Life Edit nuclease (LEG-A) and guide RNA (SGN1) targeting Huntingtin's Exon50 rs362331 'C' SNP, to be ***expressed in at least 50% of striatal neurons,*** resulting ***in at least 40% knockdown of mutant HTT protein***

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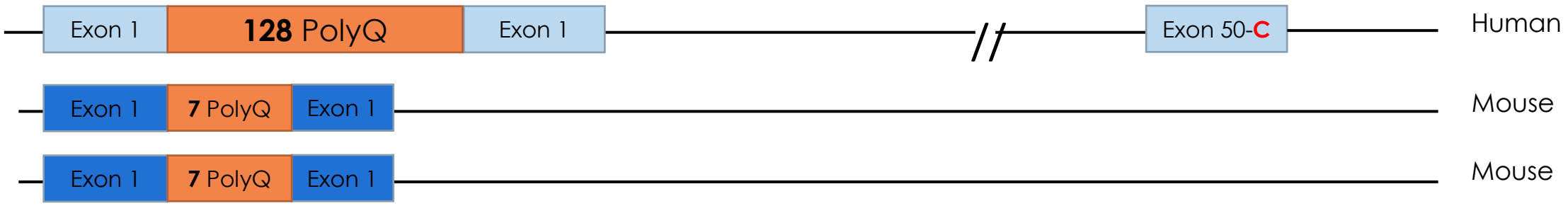
YAC128 Murine Model

Allele Selective Editing of rs362331'C' SNP with LEG-A (AAV5-LEG-A-SGN1)

Line FVB-Tg(YAC128)53Hay/J



- Humanized transgenic murine models contain two mouse alleles + full-length human Huntingtin gene including 128 CAG repeat and Exon50 rs362331 'C' SNP
- Both human mutHTT and mouse wtHTT protein are expressed (size-based differentiation)
- Licensed from University of British Columbia, Canada
- To deliver *in vivo* packaged in AAV5 (AAV5-LEG-A-SGN1)

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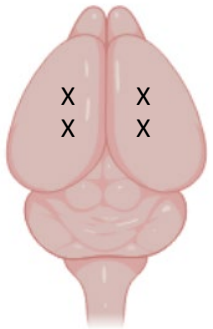
Evaluation of AAV5-LEG-A treatment at different doses and study duration

Allele Selective Editing of rs362331'C' SNP with LEG-A

Treatment	Cassette Size (bp)	Dose (vg/animal)	In-Life Duration
Naive	—	**0**	4 & 12 weeks
AAV5-CON1-LEG-A-SGN1	4518	**1.3E10**	4 & 12 weeks
AAV5-CON1-LEG-A-SGN1	4518	**6.4E10**	4 & 12 weeks
AAV5-CON1-LEG-A-SGN1	4518	**3.6E11**	4 & 12 weeks



Mouse Brain
Dorsal View

8ul injection vol.
4ul/hemisphere
2ul/injection site

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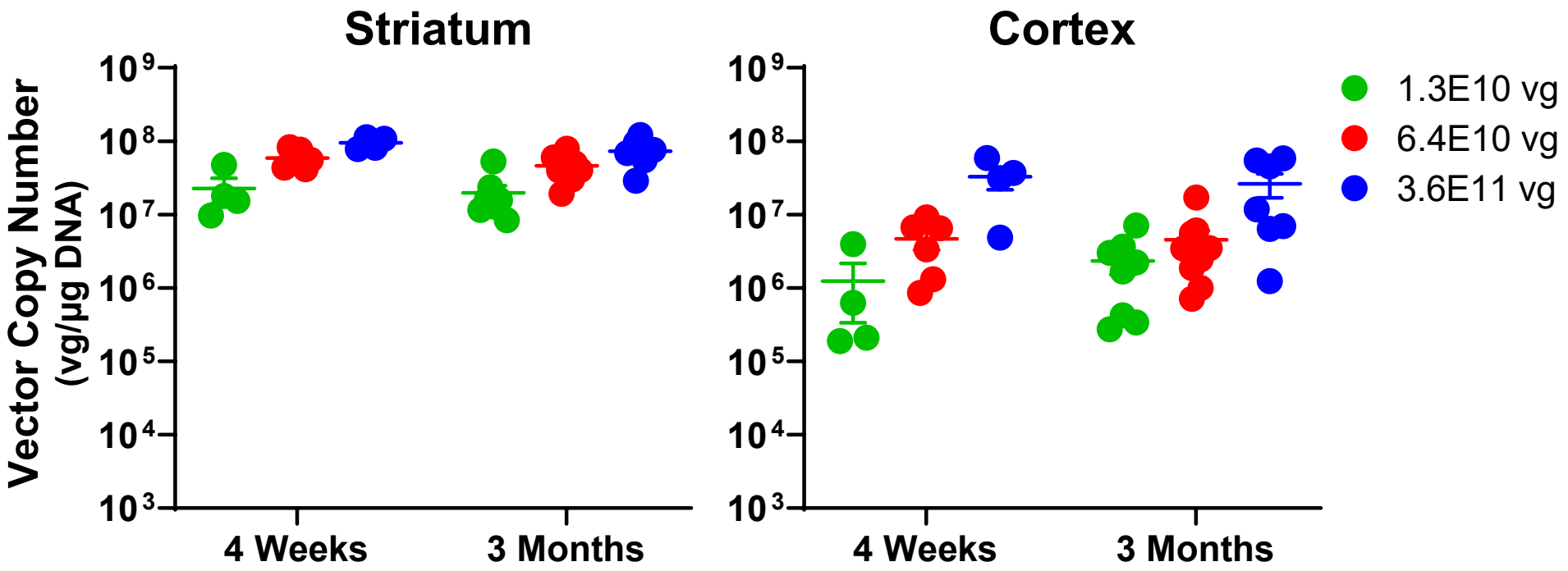
Intrastriatal AAV5-LEG-A results in high vector disposition in striatum and cortex

-Clear dose-dependence



- Each point represents individual mice with mean ± SE shown.
- Naïve cohorts are not depicted as are below LLOQ

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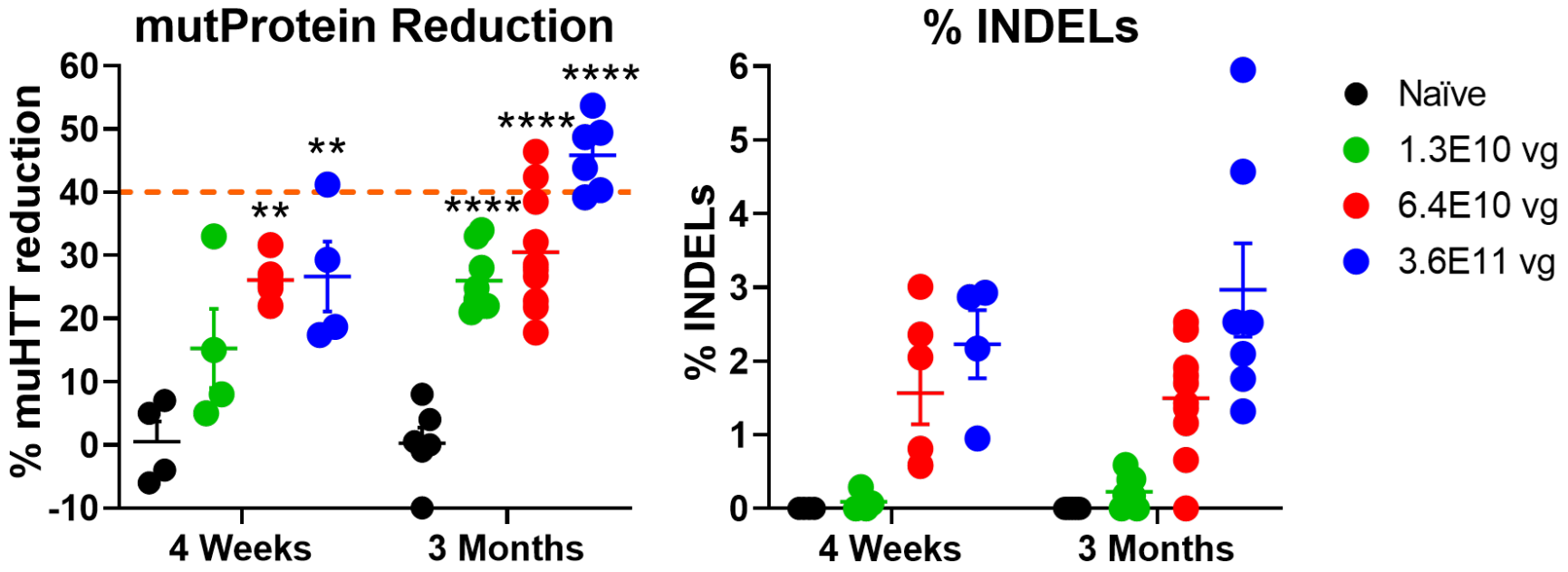
Dose-dependent reduction in striatal mutHTT protein and dose-dependent increase in INDELs at target HTT Exon50 site



- INDELs detected at target HTT Exon50 site; low rates may be due to other potential editing outcomes not detectable by short amplicon-based NGS
- Each point represents individual mice with mean ± SE shown. **P<0.01, ****P<0.0001.

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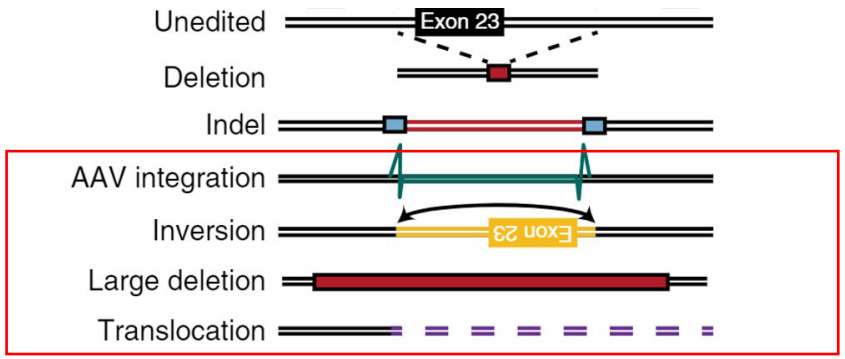
Editing of rs362331 'C' SNP with LEG-A Achieved

- AAV5-LEG-A delivered intrastriatally resulted in high dose-dependent vector disposition in YAC128 striatum and cortex
- Dose-dependent reduction in striatal mutHTT protein
  - Clinically relevant reduction in mutHTT protein observed in striatum
- Dose-dependent increase in INDELs at target HTT Exon50 site
  - Rates may be due to other potential editing outcomes not detectable by short amplicon-based NGS
  - YAC128 line contains 3 tandem copies of HTT gene
  - Some potential editing outcomes not detected with this method



Nelson et al, 2019, *Nature Medicine*

- Based on HD patient population stratification by haplotype, now focused on 'T' allele

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Allele Selective Editing of HTT SNP rs362331

AAV5-LEG-B Targeting 'T' allele

Target

AAV5-delivered Life Edit nuclease (LEG-B) and guide RNA (SGN2) targeting Huntingtin's Exon50 rs362331 'T' SNP, to be ***expressed in at least 50% of striatal neurons,*** resulting ***in at least 40% knockdown of mutant HTT protein***

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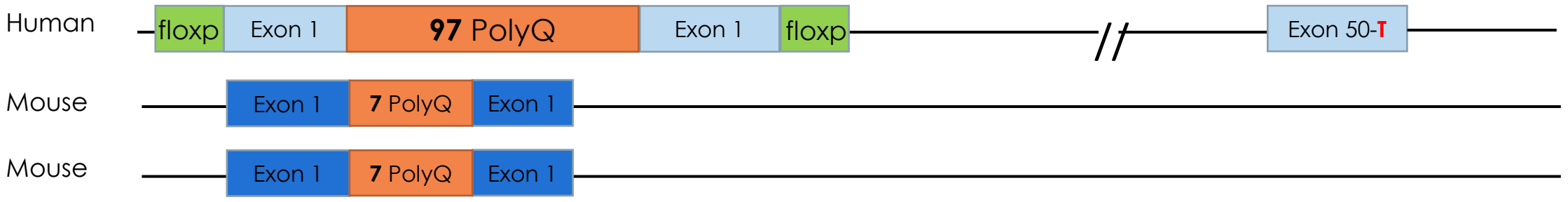
BACHD Murine Model

Allele Selective Editing of rs362331'T' SNP with LEG-B

Line FVB/N-Tg(HTT*97Q)IXwy/J



- Humanized transgenic murine models contain two mouse alleles + full-length human Huntingtin gene including 97 CAG/CAA repeat and Exon50 rs362331 'T' SNP
- Both human mutHTT and mouse wtHTT protein are expressed (size-based differentiation)
- Licensed from UCLA, USA
- To deliver *in vivo* packaged in AAV5 (AAV5-LEG-B-SGN2)

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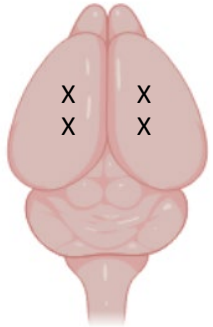
Evaluate AAV5-LEG-B treatment in BACHD mice with CON1 and TSP1 promoters

Allele Selective Editing of rs362331'T' SNP with LEG-B

Treatment	Cassette Size (bp)	Dose (vg/animal)	In-Life Duration
Naive	—	—	**4- & 12-weeks**
AAV5-CON1-LEG-B-SGN2	4736	1.72E11	**4- & 12-weeks**
AAV5-TSP1-LEG-B-SGN2	5013	2.84E11	**4- & 6-weeks**



Mouse Brain
Dorsal View

8ul injection vol.
4ul/hemisphere
2ul/injection site

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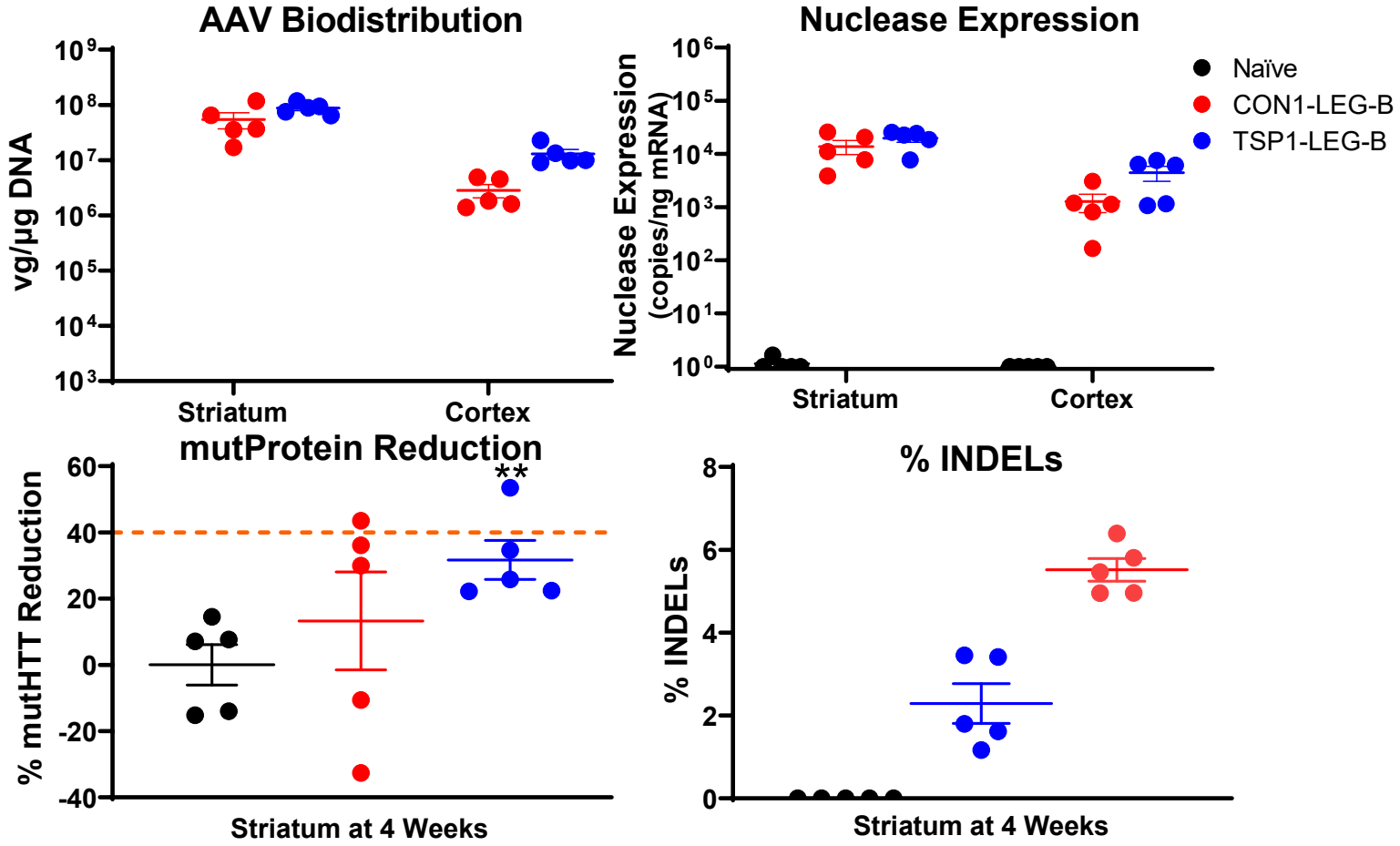
Intrastriatal AAV5-LEG-B results in high vector disposition, nuclease expression & editing at target HTT Exon50 site



- Each point represents individual mice with mean ± SE shown. **P<0.01
- Naïve vector genome cohorts are not depicted as are below LLOQ

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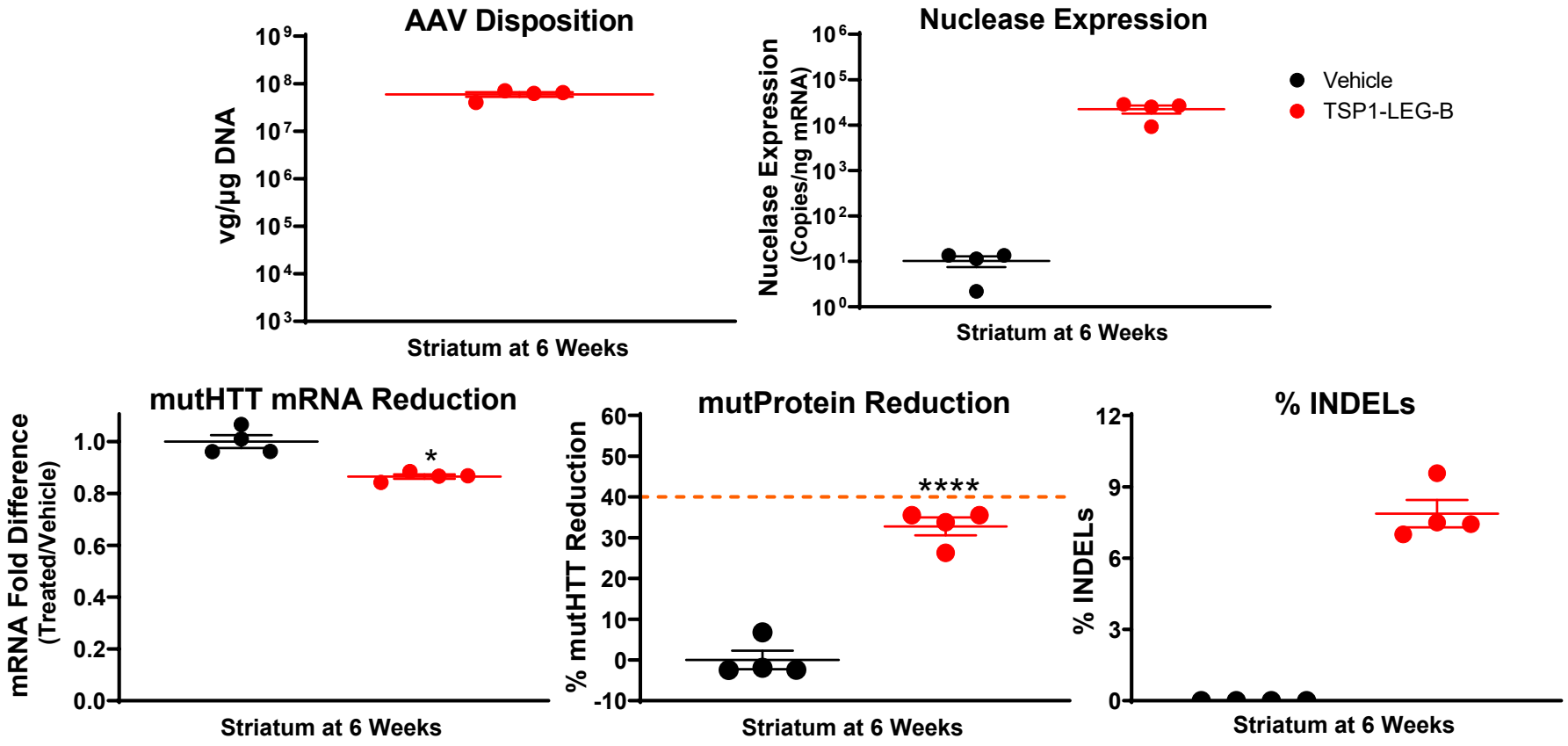
Intrastriatal AAV5-TSP1-LEG-B results in high vector disposition, nuclease expression & editing at target HTT Exon50 site



- Each point represents individual mice with mean ± SE shown. *P<0.05, ****P<0.0001
- Vehicle vector genome cohorts are not depicted as are below LLOQ

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AAV5-LEG-B cassette optimization: codon, promoters & polyA evaluation

Allele Selective Editing of rs362331'T' SNP with LEG-B

Treatment	Dose (vg/animal)	In-Life Duration
Naive	1.26E11	6-weeks
AAV5-CON1-LEG-Bmco-pA1	1.26E11	6-weeks
AAV5-TSP1-LEG-Bmco-pA1	7.32E10	6-weeks
AAV5-CON2-LEG-Bmco-pA1	7.28E10	6-weeks
AAV5-CON3-LEG-Bmco-pA1	1.48E11	6-weeks
AAV5-CON2-LEG-Bmco-pA2	1.04E11	6-weeks
AAV5-CON3-LEG-Bmco-pA2	1.29E11	6-weeks

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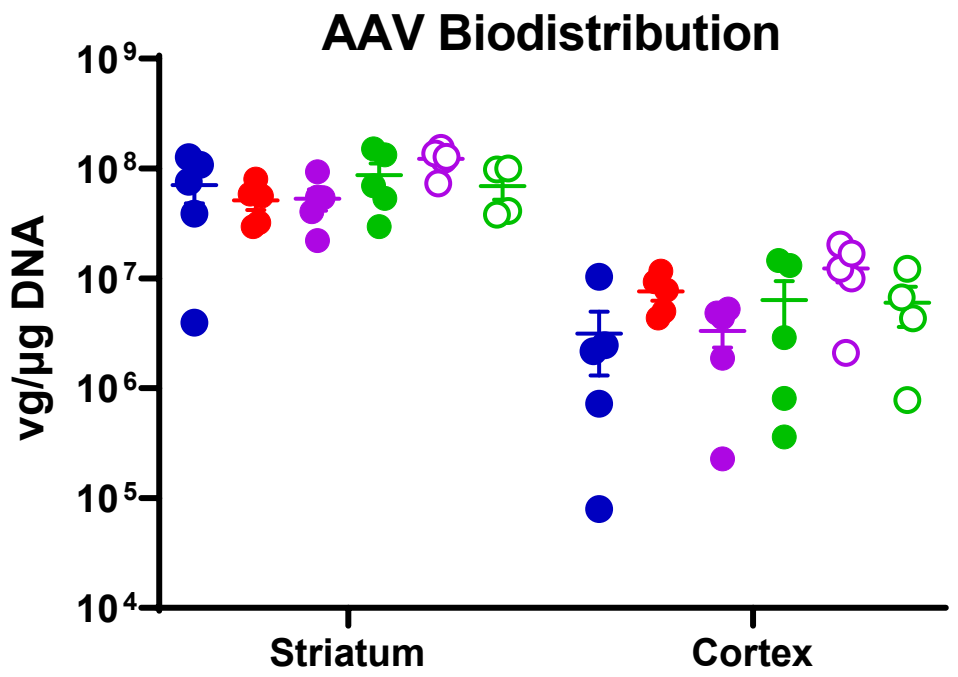
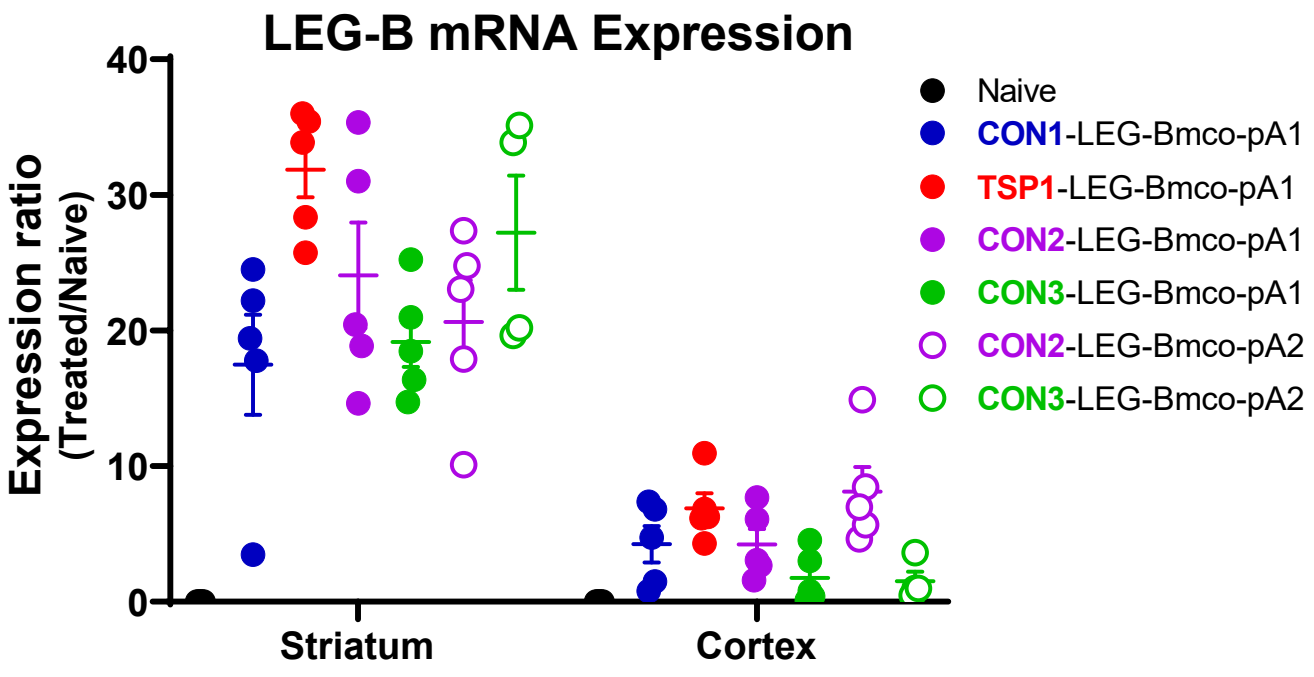
Intrastriatal AAV5-LEG-B results in high vector disposition & LEG-B Expression in striatum and cortex





- Each point represents individual mice with mean ± SE shown.
- Naïve vector genome cohorts are not depicted as are below LLOQ

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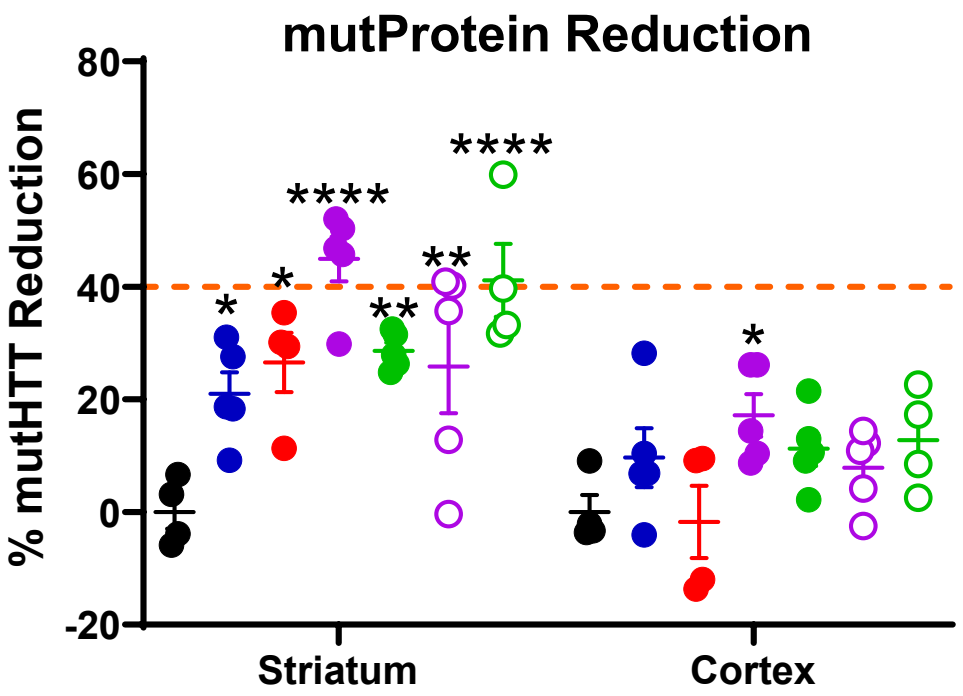
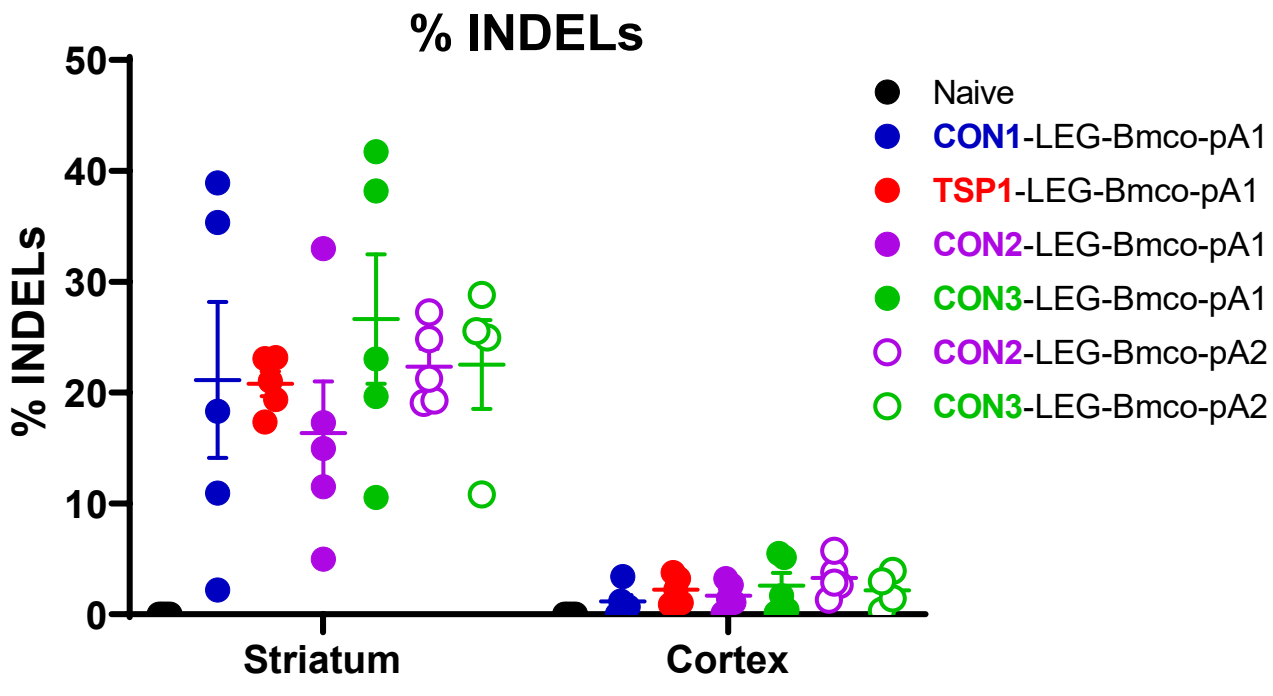
Reduction in striatal mutHTT protein & increase in INDELs at target HTT Exon50 site following intrastriatal AAV5-LEG-B administration





- Each point represents individual mice with mean ± SE shown.
- Naïve vector genome cohorts are not depicted as are below LLOQ

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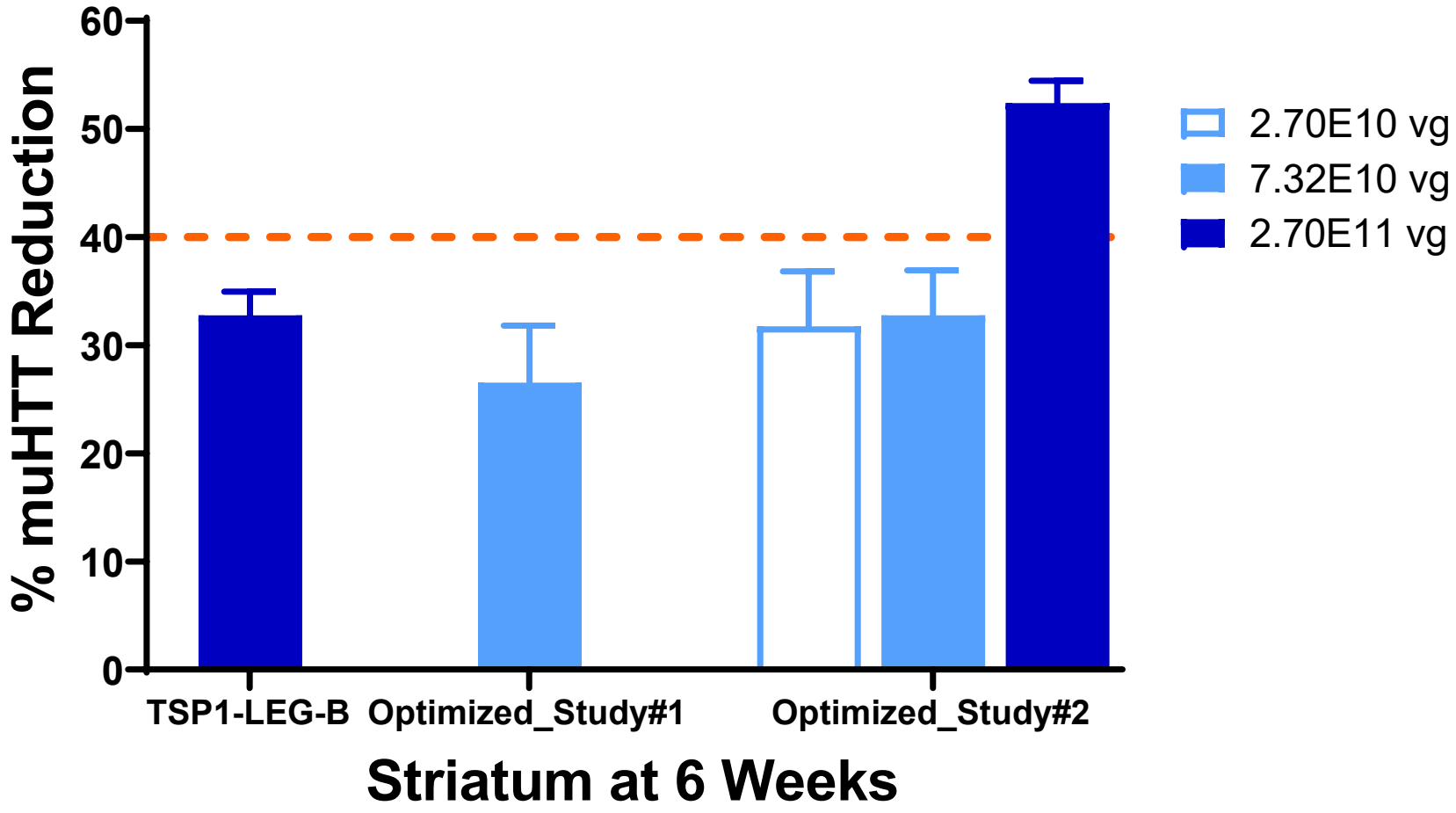
Codon optimization substantially improves activity

Evaluation of AAV5-TSP1-LEG-Bmco-pA1



Data represents mean ± SE.

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Editing of rs362331 'T' SNP with LEG-B Achieved

- AAV5-LEG-B delivered intrastriatally resulted in high dose-dependent vector disposition in BACHD transgenic mice which contain a full-length human mutant HTT gene
- Dose-dependent reduction in striatal mutHTT protein
  - Clinically relevant reduction in mutHTT protein observed in striatum
- Dose-dependent increase in INDELs at target HTT Exon50 site
- Optimization of vectors using different combinations of promoters, polyA signals, and codon optimized transgenes resulted in improved editing rates and reduction of mutant HTT protein

Conclusions

- Compact size of Life Edit nucleases facilitates all-in-one delivery with a single AAV vector characterized by diverse PAM recognition sequences that enable flexible targeting of genomic loci
- Life Edit nucleases LEG-A and LEG-B enable allele specific targeting of mutHTT based on the PAM generated by HTT Exon50 SNP rs362331
- Life Edit gene editing systems packaged into AAV5 efficiently transduce CNS tissue *in vivo*
- AAV5-delivered Life Edit gene editing systems targeting mutHTT allele resulted in clinically relevant reduction of mutHTT protein in the striatum of YAC128 and BACHD transgenic mice which contain a full-length human mutHTT gene
- Optimization of vectors using different combinations of promoters, polyA signals, and codon optimized transgenes resulted in improved editing rates and reduction of mutHTT protein
- Further evaluation is underway to identify a development candidate

Life Edit Therapeutics Poster Presentations

Group A: Huntingtin Lowering

#16

Poster #16: Allele selective SNP editing utilizing AAV5-delivered Life Edit nuclease and guide RNA resulting in meaningful reduction of mutant HTT protein

- Logan Y Brown

Group A: Huntingtin Detection/Quantification

#26

Poster #26: Mutant and wildtype huntingtin protein quantitation utilizing automated capillary electrophoresis

- Nancy Cheng

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